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(54) Title: USE OF MYCOPHENOLIC ACID, MYCOPHENOLATE MOFETIL OR DERIVATE THEREOF TO INHIBIT STENOSIS

(57) Abstract

Stenosis, particularly restenosis associated with angioplasty or cardiac bypass operations, is treated by administering a therapeutically effective amount of mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof.

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USE OF MYCOPHENOLIC ACID, MYCOPHENOLATE MOFETIL OF DERIVATE THEREOF TO INHIBIT STENOSIS

Field of the Invention

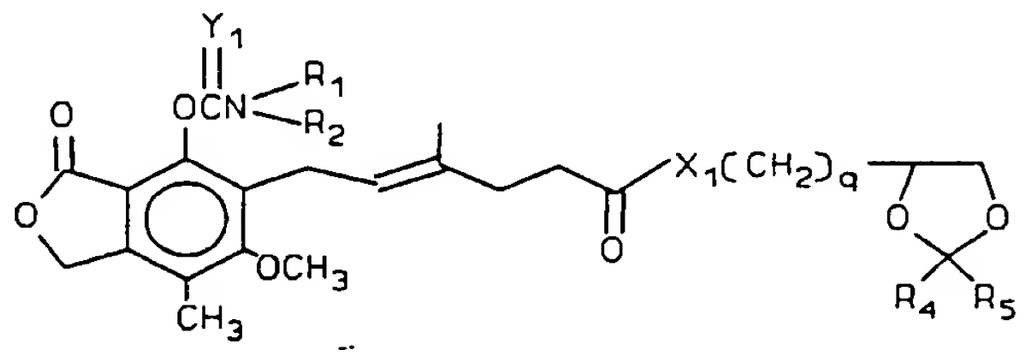
The present invention relates to methods of preventing stenosis following surgical treatment, particularly the prevention of restenosis following angioplasty through the administration of mycophenolic acid or a related compound, particularly mycophenolate mofetil.

Background Information

Mycophenolic acid is a weakly-active antibiotic found in the fermentation broth of *Pennicillium brevicompactum*. Compounds relating to mycophenolic acid, and their uses in the treatment of inflammatory diseases, autoimmune diseases, viral diseases, cancer, and/or for the prevention of allograft rejection, are disclosed in U.S. Patents Nos. 4,686,234; 4,725,622; 4,727,069; 4,748,173; 4,753,935; 4,786,637; 4,808,592; 4,861,776; 4,868,153; 4,948,793; 4,952,579; 4,959,387; and 4,922,467, all incorporated herein by reference.

Mycophenolic acid, mycophenolate mofetil, or a pharmaceuticaly acceptable salt or derivative thereof have the one of the following general structures.

A compound of Formula 1:



and the pharmaceutically acceptable salts thereof, where:

R_1 is H or lower alkyl having 1 to 6 carbon atoms;

R_2 is H, lower alkyl having 1 to 6 carbon atoms or -phenyl-4-CO_2R_3, in which R_3 is H, lower alkyl having 1 to 6 carbon atoms or a pharmaceutically acceptable cation;

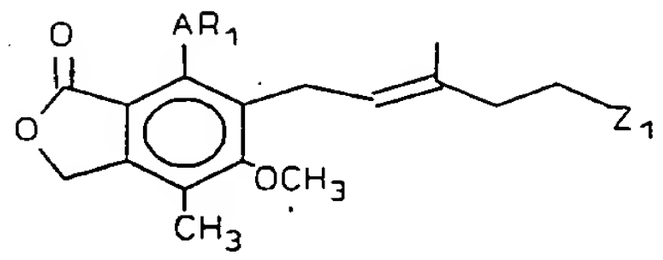
R_4 and R_5 are each independently H or lower alkyl having 1 to 6 carbon atoms;

X_1 and Y_1 are each independently O or S; and

q is an integer of 1-6.

OR

A compound of Formula 2:



and the pharmaceutically acceptable salts thereof, where:

A is oxygen or sulfur;

R_1 is selected from the group consisting of:

H, $-CR_2$, $-C(CH_2)_qCO_2R_3$ and $-C{=}C-Q_1$, in which:

A_1 is oxygen or sulfur;

q is an integer from 0-6;

R_2 is alkyl, haloalkyl or $-NR_4R_5$, where:

R_4 and R_5 are independently H, alkyl, haloalkyl, cycloalkyl, phenyl optionally monosubstituted with halogen, hydroxy, carboxy, chlorocarbonyl, sulfonylamino, nitro, cyano, phenyl, alkyl, acyl, alkoxycarbonyl, acylamino, dialkylamino or dialkylaminoethoxycarbonyl, phenyl optionally disubstituted with hydroxy, carboxy, nitro or alkyl, or benzyl optionally substituted with dialkylamino;

R_3 is H, alkyl or a pharmaceutically acceptable cation;

Q and Q_1 are independently H or $-CO_2R_3$; and

Z_1 is selected from the group consisting of: IH-tetrazolyl, $-CH_2OH$, -CHO, -CN, $-C(O)A_2R_6$ and $-C(O)NR_7R_8$, in which:

A_2 is oxygen or sulfur;

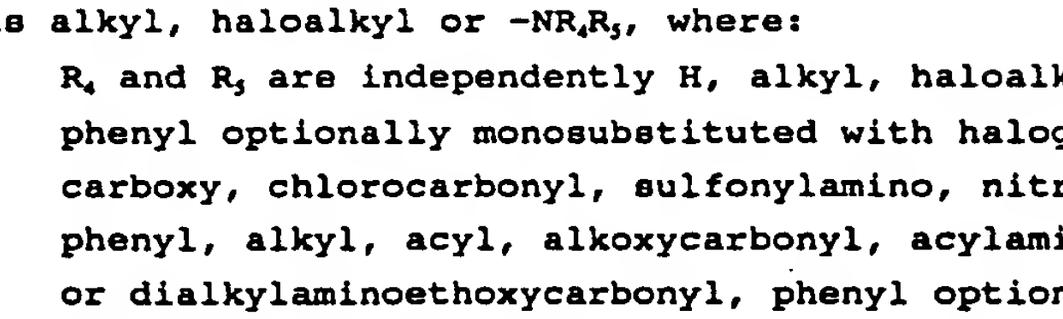
R_6 is H, alkyl, alkenyl, cycloalkyl, optionally substituted phenyl, optionally substituted benzyl or a pharmaceutically acceptable cation; and

R_7 and R_8 are independently H, alkyl or cycloalkyl, or R_7 and R_8 taken together are $-(CH_2)_2O(CH_2)_2-$, $-(CH_2)_4$, or $-(CH_2)_5-$;

with the proviso that R_1 and R_6 cannot beoth be H is A and A_2 are oxygen.

OR

A compound of Formula A:



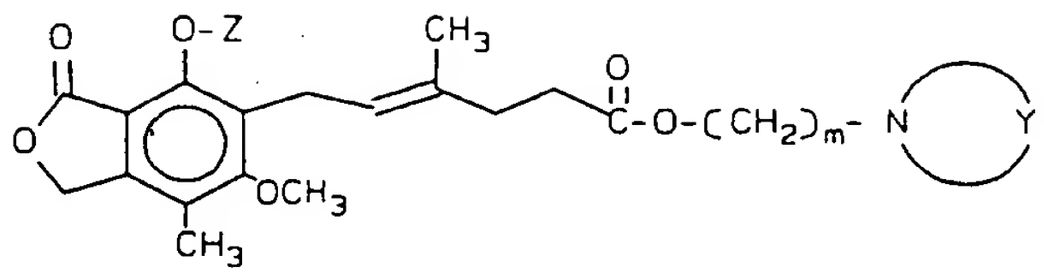
wherein Z is hydrogen or $-C(O)R$,

where R is lower alkyl or aryl,

and the pharmaceutically acceptable salts thereof.

OR

A compound of Formula I:

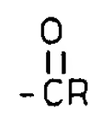


wherein:

m is an integer from two to four;

Z is selected from Formulae (a), (b), (c), or (d), as follows:

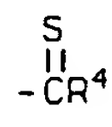
(a)



in which:

R^1 is hydrogen, alkyl having seven or more carbon atoms including cycloalkyl such as adamantyl, or $-NR^2R^3$, where R^2 is hydrogen or lower alkyl, and R^3 is hydrogen, lower alkyl, -phenyl-4-CO_2R^2 or a pharmaceutically acceptable cation;

(b)



in which:

R^4 is hydrogen, alkyl, aryl or $-NR^2R^3$;

(c)

in which:

n is an integer from zero to six, and

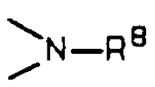
R^5 is hydrogen, lower alkyl, or a pharmaceutically acceptable cation;

(d)

in which:

R^6 and R^7 are independently hydrogen or $-CO_2R^5$; and

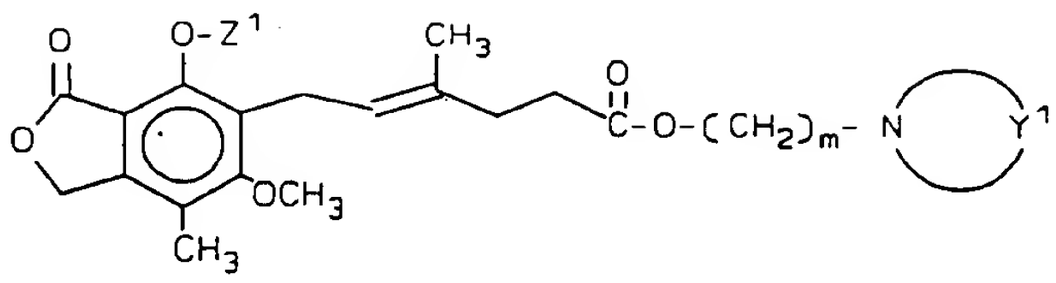
Y is lower alkylene of four to six carbon atoms, or lower alkylene of three to five carbon atoms and one member that is -O-, -S- or



where R^8 is hydrogen or alkyl of one to five carbon atoms.

OR

A compound of Formula II:

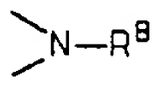


wherein:

m is an integer from two to four;

Z_1 is hydrogen or $-C(O)R^9$,

where R^9 is lower alkyl or aryl; and Y^1 is lower alkylene of four to six carbon atoms, or lower alkylene of three to five carbon atoms and one member that is -O-, -S-, or



where R^8 is hydrogen or alkyl of one to five carbon atoms; and the pharmaceutically acceptable salts thereof;

except that when m is two, Y^1 does not include $-(CH_2)_2-O-(CH_2)_2-$.

Mycophenolate mofetil, the morpholinoethyl ester of mycophenolic acid, is described in U.S. Patent No. 4,753,935 (previously incorporated by reference), and has the chemical name morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate. It has been shown effective in preventing allograft rejection, including chronic allograft rejection.

Stenosis is a narrowing of the lumen of a blood vessel caused by the thickening of a blood vessel wall, involving complex interactions between the cells of the vessel wall (connective tissue cells, especially smooth muscle cells) and circulating blood elements, with consequent restriction of blood flow. Stenosis has been associated with insult to the endothelial lining or underlying layers of the vessel wall, typically during a surgical procedure (e.g., in placing sutures through the blood vessel wall as in by-pass surgery, and during angioplasty whether by balloon, laser or otherwise). Angioplasty involves the removal of obstructions (e.g., plaque) and results in the widening of constricted blood vessels, i.e., a treatment for stenosis; the procedure often entails an insult to the endothelial lining or underlying layers, which triggers an early vascular cell proliferation, especially of smooth muscle cells (one of the cell types responding to the insult) and other connective tissue cells, and causes a thickening of the vessel wall with a corresponding narrowing of the lumen, called restenosis.

It has long been sought to provide a treatment for preventing stenosis or restenosis following surgical procedures, and has now, surprisingly, been discovered, that such treatment can be effected by the administration of an effective amount of mycophenolic acid or a related compound, particularly mycophenolate mofetil.

SUMMARY OF THE INVENTION

One aspect of the present invention concerns use of a therapeutically effective amount of mycophenolic acid or mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof for the treatment of stenosis.

Another aspect of the present invention concerns use of a therapeutically effective amount of mycophenolic acid or mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof for performing angioplasty or by-pass surgery including prophylactic administration.

Still another aspect of the present invention concerns inhibiting intimal vascular proliferation, especially of smooth muscle cells following an insult to a blood vessel wall, by administering a proliferation inhibitory amount of mycophenolic acid or mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof.

Still another aspect of the present invention is a pharmaceutical composition for the treatment of stenosis comprising a pharmaceutically acceptable non-toxic excipient and a therapeutically effective amount of mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof.

In a preferred aspect of the present invention, mycophenolate mofetil, or a pharmaceutically acceptable salt thereof, is orally administered to prevent stenosis or restenosis following angioplasty or a cardiac by-pass surgical procedure.

DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Parameters

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

As used herein, the term "alkyl" refers to a fully saturated monovalent radical containing only carbon and hydrogen, and which may be a cyclic, branched or straight chain radical. This term is further exemplified by radicals such as methyl, ethyl, t-butyl, pentyl, heptyl, pivalyl, cyclopentyl, and cyclohexyl.

The term "lower alkyl" refers to a monovalent alkyl radical of one to six carbon atoms. This term is further exemplified by such radicals as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, i-butyl (or 2-methylpropyl), isoamyl, pentyl and isopentyl.

The term "alkylene" refers to a fully saturated divalent radical containing only carbon and hydrogen, and which may be a branched or straight chain radical. This term is further exemplified by radicals such as methylene, ethylene, n-propylene, t-butylene, i-pentylene, and n-heptylene.

The term "alkoxy" refers to the group -OR wherein R is lower alkyl as herein defined.

The term "aryl" refers to a substituted or unsubstituted monovalent

unsaturated aromatic carbocyclic radical having a single ring (e.g., phenyl) or two condensed rings (e.g., naphthyl).

The term "acyl" refers to a radical based on an organic acid, e.g., $-C(O)R^1$ where R^1 is alkyl or aryl.

As used herein, the term "halo" refers to fluoro, bromo, chloro and iodo.

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography or thick-layer chromatography, or a combination of these procedures.

A "pharmaceutically acceptable salt" may be any salt derived from an inorganic or organic acid. The term "pharmaceutically acceptable anion" refers to the anion of such salts. The salt and the anion are chosen not to be biologically or otherwise undesirable. These salts are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid (giving the sulfate and bisulfate salts), nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

As used herein, the term "treatment" or "treating" means any treatment of a disease in a mammal, including:

(i) preventing the disease, that is, causing the clinical symptoms of the disease not to develop;

(ii) inhibiting the disease, that is, arresting the development of clinical symptoms; and/or

(iii) relieving the disease, that is, causing the regression of clinical symptoms.

As used herein, the terms "effective amount" or "therapeutically effective amount" means a dosage sufficient to provide treatment for the disease state being treated. This will vary depending on the patient, the disease and the treatment being effected.

As used herein, the term "stenosis" should be read to include "restenosis," except to the extent that the context or specific description indicates the contrary.

As used herein, the term "derivative" means a compound based upon the structure of mycophenolic acid bearing a substituent for -OH on the 4-position of the bicyclic ring and/or on the carboxylic acid of the side chain, as described in U.S. Patents Nos. 4,686,234; 4,725,622; 4,727,069; 4,748,173; 4,753,935; 4,786,637; 4,808,592; 4,861,776; 4,868,153; 4,948,793; 4,952,579; 4,959,387; and 4,922,467, all previously incorporated herein by reference (e.g., the groups Z and $-(CH_2)_n-N\frown Y$ in U.S. Patent No. 4,748,173).

Sources Of The Compounds Used In The Methods Of The Invention

Mycophenolic acid is available, for example, from Sigma Chemical Company, of St. Louis, Missouri.

Mycophenolate mofetil, or morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate, can be made, formulated and administered as described in U.S. Patent No. 4,753,935, previously incorporated herein by reference.

The pharmaceutically acceptable salts or derivatives of mycophenolic acid and mycophenolate mofetil can be made, formulated and administered as described in U.S. Patents Nos. 4,686,234; 4,725,622; 4,727,069; 4,748,173; 4,753,935; 4,786,637; 4,808,592; 4,861,776; 4,868,153; 4,948,793; 4,952,579; 4,959,387; and 4,922,467, all previously incorporated herein by reference.

Utility, Testing and Administration

General Utility

The compounds used in the methods of the present invention inhibit proliferating cells, including smooth muscle cells, acting through the inhibition of inosine monophosphate dehydrogenase and the consequential depletion of deoxyguanosine triphosphate, which is required for DNA synthesis and cell proliferation. The compounds are useful for preventing proliferative responses to vascular injury, e.g., stenosis following an insult to a blood vessel wall.

In particular, mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof, is administered to a patient prophylactically and/or following a surgical procedure associated with injury to the endothelium or underlying layers of a blood vessel wall (e.g., a procedure involving removal of, or damage to, endothelial cells). The compounds used in the methods of the present invention do not require the co-administration of another active agent for efficacy, although such additional active agents may be employed.

Testing

In vitro activity for treating stenosis is demonstrated by inhibiting the proliferation of smooth muscle cells. This is established by the human arterial smooth muscle cell proliferation assay. Human smooth muscle cells are grown in culture. A test group is treated with the test compound added at selected concentrations in fresh media. Both groups receive 2μCi tritiated thymidine (^{3}HTdR), a radioisotope label. After 24 hours, the cells are harvested and the amount of label incorporated into DNA is counted by scintillation; this is compared for the test and control groups, the amount being proportional to cell proliferation. Inhibition of smooth muscle proliferation is established when the test group has a lower radioisotope count than the control group. The concentrations of test compound required to inhibit proliferation by 50% (the IC_{50}), and to inhibit proliferation by more

than 95% are determined.

In vivo activity for treating stenosis is demonstrated in a rat model for arterial stenosis. A test group is treated with the test compound, starting 6 days before and continuing for 14 days after injury to the left carotid artery; the test group is compared to a control group receiving vehicle without the test compound. Injury is achieved by a gentle perfusion of air through a 10 mm long section of the left artery. The right artery is left intact. Arterial cross-sections (10 μm) are taken from both the left and right arteries of each subject, and the area of the vessel wall (endothelium, intima, media) is measured. The amount of vascular proliferation is calculated by subtracting the mean area of the intact, right carotid artery from the mean area of the injured, left carotid artery. Reduction in vascular proliferation is established when the test group shows less proliferation than the control group.

Administration

Mycophenolic acid, mycophenolate mofetil, and the pharmaceutically acceptable salts and derivatives thereof, can be administered via any of the accepted modes and formulations for agents serving similar utilities, e.g., as described in U.S. Patents Nos. 4,753,935 and 4,922,467, previously incorporated herein by reference.

Administration can be, for example, orally, nasally, parenterally or topically, in the form of solid, semi-solid, lyophilized powder, or liquid dosage forms, such as for example, tablets, suppositories, pills, capsules, powders, solutions, suspensions, emulsions, creams, lotions, aerosols, ointments, gels, or the like, preferably in unit dosage forms suitable for simple administration of precise dosages. The compositions will include a conventional pharmaceutical carrier or excipient and an active compound (mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof) and, in addition, may include other medicinal agents, pharmaceutical agents carriers, adjuvants, etc.

Generally, the compounds are administered in a therapeutically effective amount, i.e., a dosage sufficient to effect treatment, which will vary depending on the individual and condition being treated. In the present invention, the therapeutically effective amount inhibits cellular proliferative response to vascular injury. Preferably, a plasma concentration of about 0.3 μM to 10.0 μM, most preferably about 1.0 μM is therapeutically effective; this is a proliferation inhibitory amount.

The preferred manner of administration, for the conditions detailed above, is oral using a convenient daily dosage regimen which can be adjusted according to the degree of affliction. For such oral administration, a pharmaceutically acceptable, non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate and the like. Such compositions take the form of

solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like.

Preferably the compositions will take the form of a pill or tablet and thus the composition will contain, along with the active ingredient, a diluent such as lactose, sucrose, dicalcium phosphate, and the like; a disintegrant such as starch or derivatives thereof; a lubricant such as magnesium stearate and the like; and a binder such as starch, gum acacia, polyvinylpyrrolidone, gelatin, cellulose and derivatives thereof, and the like.

Liquid pharmaceutically administerable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound (about 0.5% to about 20%), as described above, and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol and the like, to thereby form a solution or suspension.

If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, 16th Ed., (Mack Publishing Company, Easton, Pennsylvania, 1980). The composition to be administered will, in any event, contain a quantity of the active compound(s) in a pharmaceutically effective amount for relief of the particular condition being treated when administered in accordance with the teachings of this invention.

A therapeutically effective daily oral dose is from as low as 0.02 mg/kg to about 100 mg/kg of body weight, preferably from about 25 mg/kg to about 60 mg/kg. Intravenous doses are comparable. Mycophenolate mofetil is administered for preventing allograft rejection in oral dosages of 2.0, 3.0, 3.5 and 4.0 grams per day, corresponding to a daily dosage from about 25 mg/kg to about 60 mg/kg, depending upon the patient and the allograft being treated. Similar dosing regimens are effective in the methods of treatment of the present invention.

EXAMPLES

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

EXAMPLE 1

This example illustrates the preparation of a representative pharmaceutical formulation for oral administration containing an active compound, e.g., mycophenolic acid, mychophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-

methyl-4-hexenoate hydrochloride.

Ingredients	Quantity per Capsule, mgs.
Active compound	200
lactose, spray-dried	148
magnesium stearate	2

The above ingredients are mixed and introduced into a hard-shell gelatin capsule.

EXAMPLE 2

This example illustrates the preparation of another representative pharmaceutical formulation for oral administration containing an active compound, e.g., mycophenolic acid, mychophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

Ingredients	Quantity per Capsule, mgs.
Active compound	400
cornstarch	50
lactose	145
magnesium stearate	5

The above ingredients are mixed intimately and pressed into single scored tablets.

EXAMPLE 3

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound, e.g., mycophenolic acid, mychophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

Ingredients	
Active compound	1.0 g.
fumaric acid	0.5 g.
sodium chloride	2.0 g.
methyl paraben	0.1 g.
granulated sugar	25.5 g.
sorbitol (70% solution)	12.85 g.
Veegum K (Vanderbilt Co.)	1.0 g.
flavoring	0.035 ml
colorings	0.5 mg
distilled water	q.s. to 100 ml

EXAMPLE 4

Determination of *In Vitro* Activity Utilizing Human Arterial Smooth Muscle Proliferation Assay

Human smooth muscle cells, AG11545 (obtained from Coriell Cell Repository) were plated at low concentration (2.5×10^4 cells/ml) in 24-well plates (Dulbecco's modified Eagle's medium containing 10% fetal calf serum). The cells were allowed to grow for 48 hours. Fresh media containing mycophenolic acid (0.01 μM, 0.1 μM, 1.0 μM and 10 μM) was then added (except to the control wells). A label of 2μCi ^{3}HTdR/well was also added. The cells were allowed to grow for 24 hours, and then harvested using TCA precipitation. The amount of label incorporated into DNA was counted by scintillation, and compared for test and control wells. From the results, the *in vitro* concentration of mycophenolic acid effective for reducing smooth muscle cell proliferation by 50% (IC_{50}), and by more than 95%, were determined.

When tested by this method, mycophenolic acid had an IC_{50} of about 0.3 μM. Concentrations of 1.0 μM inhibited smooth muscle cell proliferation by more than 95%. This is predictive that mycophenolic acid is useful for inhibiting stenosis or restenosis in human patients undergoing surgical procedures.

By following the same procedure and substituting pharmaceutically acceptable salt or non-ester derivative of mycophenolic acid for mycophenolic acid, there is obtained similar activity in reducing smooth muscle cell proliferation. This is predictive that the pharmaceutically acceptable salts and non-ester derivatives of mycophenolic acid are useful for inhibiting stenosis or restenosis in human patients undergoing surgical procedures.

Mycophenolic acid concentrations of 0.3 to 10 μM are readily attainable in humans treated with daily oral doses of about 25 to about 60 mg/kg of mycophenolate mofetil. This is predictive that mycophenolate mofetil, its pharmaceutically acceptable salts and the ester derivatives of mycophenolic acid are useful for inhibiting stenosis or restenosis in human patients undergoing surgical procedures.

EXAMPLE 5

Determination of *In Vivo* Activity Utilizing The Rat Arterial Stenosis Model

Test Materials

Mycophenolate mofetil is suspended in SSV, a vehicle consisting of 0.5% sodium carboxymethylcellulose, 0.9% NaCl, 0.4% Tween 80, and 0.9% benzyl alcohol in water.

Animals

Male Sprague-Dawley rats (Crl, CD® (SD) BR), 3-4 months old and weighing 350±25 grams, were used. The animals were housed individually and fed

standard rodent chow.

Treatment Regimen

An experimental group of 30 rats was treated orally with a daily dose of 30 mg/kg of mycophenolate mofetil, divided into 2 equal doses, given 6 hours apart. Treatments began 6 days before and continued for 14 days after injuring the carotid artery to induce neointimal proliferation. A control group of 30 rats was similarly treated with the SSV vehicle alone.

Arterial Injury Model

The left carotid artery of each rat was injured using the technique described by Fishman, JA, et al., "Endothelial regeneration in the rat carotid artery and the significance of endothelial denudation in the pathogenesis of myointimal thickening," Lab Invest., 1975, 32:339-351.

Each animal was anesthetized by administering ip a mixture of 58% ketamine (Fort Dodge Laboratories, Iowa) 60 mg/kg, and 42% xylazine (Lloyd Laboratories, Iowa) 10 mg/kg. A 10 mm long section of the distal left common carotid artery was then exposed. Silk ties were positioned and loosely ligated at each end of the exposed artery. A puncture site was created near each ligature, using a 30-gauge needle attached to a syringe containing Tyrode's solution (Sigma). Next, Tyrode's solution was perfused through these orifices to rinse blood from the isolated vessel. Air was then gently perfused through the same orifices at a rate of 25 ml/min for 3 minutes, to injure the artery. The puncture sites were allowed to clot, the ligatures were removed, and the wound was closed. This procedure produced endothelial denudation as well as some disruption to the underlying intima and media of the vessel wall.

Fourteen days after injury, the animals were sacrificed. Both the left and right carotid arteries were removed to 10% formalin and then to 30% sucrose, three days before mounting and scoring for neointimal proliferation.

Analysis of Neointimal Proliferation

The recovered arteries were cut into quarters and the 4 segments embedded together in a single block of Optimum Cutting Temperature (O.T.C.) compound (Miles, Indiana). From the block, 15 arterial cross sections, each 10 μm thick, were sliced with a microtome/cryostat (Miles, Indiana) at -20°C and than stained with hematoxylin-eosin. The stained arterial cross-sections were projected through a microscope onto a digitizing board connected to a computer programmed with SigmaScan (Jandel Scientific, Corte Madera, CA). Using an electronic pen to draw around the projected inner and outer arterial wall, the mean cross-sectional area of both the left (injured) and right (control) arteries were computed electronically from sections 1, 4, 7, 10, and 13 of the 15 arterial cross-sections. The final area of smooth muscle proliferation for each rat was calculated by subtracting the mean area of the control artery from that of the injured artery. All scoring was done blindly, with the investigator unaware of whether the cross-section came from a test or control rat, or from an injured or intact artery.

Statistical Analysis

Both a parametric unpaired t-test and a nonparametric Mann Whitney U test were used to compare the mycophenolate mofetil-treated group to the SSV vehicle-treated control group, to determine whether there was significant reduction in mean lesion size.

Results

Mycophenolate mofetil at 30 mg/kg markedly reduced the vascular proliferative response to air-perfusion-induced vessel wall injury at Day 14 after injury, giving the following mean cross sectional areas:

	Injured Artery	Intact Artery
Mycophenolate mofetil	1772 ± 295 mm^2	1176 ± 103 mm^2
SSV vehicle control	2289 ± 532 mm^2	1186 ± 69 mm^2

giving a mean area of vascular proliferation (subtracting area of injured artery from area of intact artery) of:

	Vascular Proliferation
Mycophenolate mofetil	546 ± 280 mm^2
SSV vehicle control	1103 ± 533 mm^2

corresponding to a 51% reduction in neointimal proliferation ($p < 0.05$).

Conclusion

Mycophenolate mofetil inhibits neointimal proliferation by 51% following vascular injury *in vivo*. This is predictive that mycophenolate mofetil is useful for inhibiting stenosis or restenosis in human patients undergoing surgical procedures.

By repeating the procedure and substituting mycophenolic acid or a pharmaceutically acceptable salt or derivative thereof, or a pharmaceutically acceptable salt or derivative of mycophenolate mofetil, for mycophenolate mofetil, similar inhibition of neointimal proliferation is observed. Mycophenolic acid and the pharmaceutically acceptable salts and derivatives thereof, and the pharmaceutically acceptable salts and derivatives of mycophenolate mofetil, are useful for inhibiting stenosis or restenosis in human patients undergoing surgical procedures.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

WHAT IS CLAIMED IS:

1. Use of a therapeutically effective amount of mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof to inhibit stenosis in a mammal in need thereof.

2. The use of Claim 1 comprising administering mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof, to an angioplasty or cardiac by-pass patient.

3. The use of Claim 2 comprising administering mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof, prophylactic to the angioplasty or cardiac by-pass procedure.

4. The use of Claim 3 comprising the administration of mycophenolate mofetil or a pharmaceutically acceptable salt thereof.

5. The use of Claim 1 consisting of the administration of mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof.

6. The use of Claim 1 consisting of the administration of mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt thereof.

7. Use of a proliferation inhibitory amount of mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof to inhibit the proliferation of vascular cells following an insult to a vessel wall in a mammal in need thereof.

8. The use of Claim 7 comprising inhibiting the proliferation of smooth muscle cells.

9. The use of Claim 7 wherein said proliferation inhibitory amount is a plasma concentration of about 0.3 μM to about 10.0 μM.

10. The use of Claim 9 comprising the oral administration of mycophenolate mofetil in a therapeutically effective amount to give a proliferation inhibitory amount of mycophenolic acid.

11. The use of Claim 10 comprising the oral administration of about 25 mg/kg to about 60 mg/kg of mycophenolate mofetil.

12. Use of a therapeutically effective amount of mycophenolate mofetil, or a pharmaceutically acceptable salt thereof for treatment to inhibit stenosis in a mammal.

13. The use of Claim 12 wherein said therapeutically effective amount is about 25 mg/kg/day to about 60 mg/kg/day.

14. The use of Claim 13 wherein mycophenolate mofetil or a pharmaceutically acceptable salt thereof is administered orally.

15. A pharmaceutical composition for the treatment of stenosis comprising a pharmaceutically acceptable non-toxic excipient and a therapeutically effective amount of mycophenolic acid, mycophenolate mofetil, or a pharmaceutically acceptable salt or derivative thereof

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/09932

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 A61K31/535; A61K31/365

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
T	TRANSPLANTATION PROCEEDINGS vol. 25, no. 1, 1993, pages 770 - 771 C.R. GREGORY ET AL. 'EFFECTS OF TREATMENT WITH CYCLOSPORINE, FK 506, RAPAMYCIN, MYCOPHENOLIC ACID, OR DEOXYSPERGUALIN ON VASCULAR MUSCLE PROLIFERATION IN VITRO AND IN VIVO' see the whole document ---	1-15
X	SURGICAL FORUM vol. 43, October 1992, pages 383 - 385 D.M. STEELE ET AL. 'RS-61443 INHIBITS INTIMAL HYPERPLASIA IN AORTIC ALLOGRAFTS' see the whole document --- -/--	1-15

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 JULY 1993	26.07.93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HOFF P.J.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	FASEB vol. J6, no. 4, April 1992, page A940 C. GREGORY ET AL. 'USE OF ANTIPROLIFERATIVE AGENTS FOR THE TREATMENT OF OCCLUSIVE VASCULAR DISEASE' see the whole document	1-3,5-8, 15
A	'THE MERCK MANUAL' 1987 , MERCK SHARP & DOHME RESEARCH LABORATORIES , RAHWAY, N.J. CORONARY ARTERIAL BYPASS SURGERY AND ANGIOPLASTY see page 483 - page 484	2-3
A	EP,A,0 281 713 (SYNTEX) 14 September 1988 cited in the application	1-14
X	see abstract; claims	15
A	US,A,4 686 234 (NELSON ET AL.) 11 August 1987 cited in the application	1-14
X	see abstract; claims	15
A	US,A,4 725 622 (NELSON ET AL.) 16 February 1988 cited in the application	1-14
X	see abstract; claims	15
A	US,A,4 727 069 (NELSON ET AL.) 23 February 1988 cited in the application	1-14
X	see abstract; claims	15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09932

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 1-14 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9209932
SA 67967

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 12/07/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0281713	14-09-88	US-A-	4753935	28-06-88
		AU-B-	599728	26-07-90
		AU-A-	8254087	04-08-88
		DE-A-	3773660	14-11-91
		JP-A-	63188672	04-08-88
		US-A-	4808592	28-02-89
		US-A-	4786637	22-11-88
		US-A-	4868153	19-09-89
		US-A-	4952579	28-08-90
		US-A-	4948793	14-08-90
		US-A-	4992467	12-02-91
US-A-4686234	11-08-87	None		
US-A-4725622	16-02-88	US-A-	4959387	25-09-90
US-A-4727069	23-02-88	US-A-	4748173	31-05-88
		US-A-	4861776	29-08-89
		US-A-	5177072	05-01-93